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RESPIRATORY, ACID–BASE AND IONIC STATUS OF KEMP'S RIDLEY SEA TURTLES (*LEPIDOCHELYS KEMPI*) SUBJECTED TO TRAWLING

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Abstract—1. Kemp's ridley sea turtles were placed in a commercial shrimp trawl equipped with a turtle excluder device (TED), resulting in a burst of apneic swimming and a brief bout of forced submergence ($\leqslant 7.3$ min).

2. Trawl tests induced a significant non-respiratory (metabolic) acidosis; blood pH declined almost 0.4 units and plasma [lactate] increased 6-fold from pre- to post-trawl conditions.

3. Significant changes in blood parameters occurred regardless of the duration of forced submergence (range 2.7–7.3 min), suggesting apneic activity was a significant contributor to the observed acid–base imbalance.

INTRODUCTION

Sea turtles are among the largest and most active of extant reptiles. Their correspondent metabolic O_2 demands are high, when compared to other reptiles (Glass and Wood, 1983; Wood *et al.*, 1984; Jackson, 1985). Sea turtles are also excellent breath-hold divers, capable of prolonged submergence (20–180 min) (Lutz and Bentley, 1985) and of dives to substantial depths (300–1,000 m) (Eckert *et al.*, 1986; Kooyman, 1988). Respiration and ventilation has been studied in sea turtles when resting or exercising on land (Tenney *et al.*, 1974; Jackson and Prange, 1979) and when resting, swimming, or forcibly submerged in water (Berkson, 1966; Prange, 1976; Butler *et al.*, 1984; Lutz *et al.*, 1989). The majority of previous work has focussed on green sea turtles (*Chelonia mydas*) and loggerhead sea turtles (*Caretta caretta*). Less information is available for other species of sea turtle.

The present study examined respiratory effects of forced submergence in Kemp's ridley sea turtles (*Lepidochelys kempi*). *L. kempi*, like other sea turtle species, are subject to incidental capture and, hence, forced submergence in fishing nets. It has been estimated, for instance, that approximately 11,000 sea turtles are drowned annually in commercial shrimp trawls in the Gulf of Mexico/Atlantic Ocean (Henwood and Stuntz, 1987). The present study was undertaken specifically to determine the effects of "trawl stress" on the blood gas, acid–base, and ionic status of Kemp's ridley turtles. Turtles were placed in a commercial shrimp trawl equipped with a turtle excluder device (TED). Trawl tests induced a brief bout of forced submergence ($\leqslant 7.3$ min) before turtles escaped the trawl via the TED or were released manually by a SCUBA diver. This period of forced submergence was comparable in duration to voluntary dives in green and loggerhead sea turtles (Lutz and Bentley, 1985). A preliminary account of this work has appeared elsewhere (Stabenau and Heming, 1989).

MATERIALS AND METHODS

Turtles

Experimental animals were part of a group of Kemp's ridley turtles utilized in TED certification tests conducted by the National Marine Fisheries Service (NMFS). The 2- or 3-year old turtles exhibited an average straight-line carapace length and body weight of 39.4 cm and 5.4 kg, and 46.8 cm and 16.5 kg, respectively. Front flipper tags provided individual identification.

The turtles had been reared from hatchlings at the NMFS Galveston Laboratory (Texas), as described by Fontaine *et al.* (1989). The animals were conditioned to swimming, prior to use in the present tests. Conditioning involved 10 min of exercise in a flow chamber (Stabenau, 1988) at a swimming speed of 42 cm/sec, 6 times per week for 3 months. Turtles then were placed in 0.4-hectare ponds (Sea Arama Marineland, Galveston, Texas) for 3 weeks. Thereafter, the animals were transported by aircraft to the NMFS Panama City Laboratory in Florida, where they were maintained in a triangular pen (24 × 18 × 24 m) in St. Andrews Bay for at least 24 hr prior to the test.

Protocol of trawl tests

Trawl tests were conducted aboard a single-rigged commercial shrimp trawler (F/V MISS CARRIE, 22 m) outfitted with a TED. Trawling speed averaged 128 cm/sec. Water and turtle cloacal temperatures were measured immediately prior to each test. Test animals were transferred from the water's surface to the trawl by means of a messenger line attached to the center of the trawl headrope. Each turtle was placed inside a weighted bag (64 × 64 cm) at the surface, attached to the messenger line with a snap clip, and sent underwater to SCUBA divers stationed on the trawl. Turtles were released into the mouth of the trawl by placing them under and behind the trawl headrope.

Each turtle was given 5 min to escape after its release into the trawl. Turtles remaining in the trawl at the end of the 5-min period were removed by a SCUBA diver and released. A total of 2.25 min was added to the time in the trawl to account for the additional time each turtle spent underwater. This value was based on the following maximum estimates: 1 min for the turtle to reach the SCUBA diver at the trawl headrope; 1 min to introduce the turtle into the trawl; 15 sec for the turtle to reach the surface after its release or escape from the trawl. Thus, the maximum time any turtle spent underwater was approximately 7.3 min.

Turtles were immediately recaptured at the surface and returned to the trawler. All turtles were released into the wild at the conclusion of the tests.

Blood sampling and analyses

Venous blood samples were collected from the cervical sinus, according to procedures described by Owens and Ruiz (1980). Pre-trawl samples were collected from one sinus immediately prior to placement of each turtle in the weighted bag. Post-trawl samples were collected from the opposing sinus within 1 min of turtle recapture at the surface. During each blood collection, a 1-ml sample was collected anaerobically with a heparinized, gas-tight syringe for analyses of gases and pH, and a 2-ml sample was collected with a heparinized vacutainer for analyses of hematocrit and ions.

Samples for blood gas and pH determinations were stored on ice until analysed. It was not possible for us to measure blood gases and pH at the turtle body temperature (27°C) under conditions of the trawl tests. Instead, blood pH, pCO_2, pO_2, and $[HCO_3^-]$ were determined at 37°C (Corning blood gas/pH analyser, model 278) and then back-corrected to the turtle body temperature (27°C) using techniques of Kelman and Nunn (1966). Although these temperature-corrected data cannot be regarded as quantitatively exact, they do provide valid qualitative information on the effects of trawl stress.

For lactate determinations, a 200-μl aliquot of plasma was obtained by centrifugation, deproteinated by addition of 400 μl of chilled 8% perchloric acid followed by centrifugation, and the resultant supernatant stored frozen for later analysis. [Lactate] was determined enzymatically (Sigma, kit 826-UV). An additional aliquot of plasma was stored frozen for later analysis of K^+, Na^+, and Cl^-. $[Na^+]$ and $[K^+]$ were determined by flame photometry (Jenway, model PFP7). $[Cl^-]$ was determined by electrometric titration (Haake Buchler Instruments, model 4425000).

No discernable differences were evident between data from 2- and 3-year old turtles or between data from turtles escaping the trawl on their own and animals released manually (Student's t-tests, $P > 0.05$). The data were summarized, therefore, without regard to turtle age or the means of trawl escape. Differences between pre- and post-trawl data of individual turtles were assessed with paired Student's t-tests. Mean differences associated with a probability of ≤ 0.05 were regarded as significantly different from zero.

Assessment of handling and transport effects

To enable assessment of the potential effects of animal transport to the trawl test site, pre-transport blood samples were collected and analysed while the turtles were at the NMFS Galveston Laboratory. Blood sample collection and analyses were identical to that described for the trawl test, with the following exceptions. Blood pH was determined at 30°C, the turtle body temperature at the time of sampling (Radiometer, electrode type G297/G2). The total CO_2 content of anaerobically-obtained plasma was measured, using the CO_2 electrode method of Cameron (1971) (Radiometer, electrode type E5036). $[HCO_3^-]$ and pCO_2 were calculated, using rearrangements of the Henderson–Hasselbalch equation for carbonic acid and experimentally-derived values for αCO_2 (38.8 nmol/L torr) and pKa (6.17) of plasma of Kemp's ridley turtles at 30°C and pH 7.4–7.5 (Stabenau and Heming, unpublished data).

To assess the effects of handling, yearling turtles were placed prone in weighted bags for 5 min without submergence. Blood samples were collected before and after the turtles were confined in this way. Sample collection and analyses were identical to those described for the pre-transport study, with the exception that lactate, hematocrit, and ion levels were not determined.

RESULTS

Effects of animal handling and transport

Table 1 gives the blood characteristics of Kemp's ridley turtles prior to animal transport to the trawl test site and determined, where applicable, at the turtle body temperature (30°C). Transport effects were ascertained by comparing pre-transport data (Table 1) with data from post-transport, pre-trawl turtles (Table 2). Transport of turtles had a slight effect on blood characteristics (Tables 1 and 2). There was reasonable agreement between the blood gas and pH data for turtles prior to and after transport, given the differences in body temperature (30 vs 27°C) and the questionable validity of using clinical (human) temperature-correction factors for the blood gases and pH of samples collected at the trawl test site. The requisite correction factors, however, are not available in the literature for Kemp's ridley turtles. Plasma [lactate] increased marginally post-transport, but remained within the range of values reported for control animals of other sea turtle species (Berkson, 1966; Hochachka *et al.*, 1975; Wood *et al.*, 1984; Lutz and Bentley, 1985). Plasma $[Cl^-]$ decreased significantly post-transport, while plasma $[K^+]$ increased. Hematocrit and plasma $[Na^+]$ were unaffected by transport.

In studies designed to mimic the handling experienced by turtles during trawl tests, short-term confinement in weighted bags was found to have no discernable affect on the respiratory and acid–base status of yearling turtles (Table 3). Moreover, pre- and post-confinement pH, pCO_2, and $[HCO_3^-]$ (Table 3) resembled the pre-transport data of 2- and 3-year old turtles (Table 1). This suggests the changes observed during trawl tests were the result of trawl stress, rather than an artifact of handling.

Effects of trawl stress

Trawl stress was associated with the development of a significant blood acidosis. Blood pH declined an average of 0.37 units from pre-trawl to post-trawl conditions (Table 2). This acidosis had a large

Table 1. Respiratory, acid–base, and ionic status (mean ± SE, $N = 7$) of Kemp's ridley sea turtles at 30°C prior to transport to the trawl site

Blood pH	7.45 ± 0.02	Blood pCO_2, torr	43.2 ± 2.8
Blood $[HCO_3^-]$, mM	32.6 ± 1.3	Plasma [lactate], mM	0.7 ± 0.1
Hematocrit, %	30.8 ± 1.2	Plasma $[Na^+]$, mM	140.5 ± 3.9
Plasma $[Cl^-]$, mM	112.2 ± 2.0	Plasma $[K^+]$, mM	6.3 ± 0.5

Table 2. Effects of trawl stress on the respiratory, acid–base, and ionic status (mean ± SE, $N = 14$–17) of Kemp's ridley sea turtles at 27°C

Characteristic	Pre-trawl	Post-trawl	Difference (Post-Pre)
Blood pH	7.40 ± 0.03	7.04 ± 0.02	−0.37 ± 0.02*
Blood pO_2, torr	29.4 ± 1.5	28.7 ± 1.5	−0.7 ± 1.5
Blood pCO_2, torr	40.9 ± 3.1	53.7 ± 3.7	12.8 ± 3.3*
Blood $[HCO_3^-]$, mM	25.7 ± 0.8	12.1 ± 0.7	−13.6 ± 1.1*
Plasma [lactate], mM	1.7 ± 0.3	10.2 ± 0.4	8.5 ± 0.4*
Hematocrit, %	31.0 ± 1.0	31.1 ± 1.1	0.1 ± 1.0
Plasma $[Na^+]$, mM	141.7 ± 3.8	141.0 ± 2.6	−0.7 ± 5.2
Plasma $[Cl^-]$, mM	93.5 ± 1.8	93.8 ± 3.4	−0.3 ± 3.4
Plasma $[K^+]$, mM	8.7 ± 0.3	12.9 ± 0.6	4.2 ± 0.6*

*Significantly different from zero (paired t-test, $P \leqslant 0.05$).

non-respiratory component, as indicated by the 6-fold increase in plasma [lactate] (Table 2). The submergence time of individual turtles ranged from 2.7 to 7.3 min. Observed changes in blood pH and plasma [lactate] were not statistically correlated with submergence time (Fig. 1) ($r = 0.004$ in both cases). Blood pCO_2 increased an average of 12.8 torr during trawl tests, while $[HCO_3^-]$ declined 13.6 mM (Table 2). There was no discernable change in hematocrit during trawl tests. Similarly, trawl stress had no effect on plasma $[Na^+]$ or $[Cl^-]$ (Table 2). However, post-trawl $[K^+]$ was significantly increased.

DISCUSSION

Trawl stress induced a significant blood acidosis in Kemp's ridley sea turtles. This acidosis originated primarily from non-respiratory (metabolic) sources. The 0.37-unit decrement in blood pH between pre-trawl and post-trawl samples was accompanied by a 8.5-mM increase in plasma [lactate]. Given a true plasma buffering capacity (β_{tp}) of Kemp's ridley turtles of 22.4 mM/pH unit at 30°C and 31% hematocrit (Stabenau and Heming, unpublished data), the data yield a proton-lactate deficit ($\beta_{tp} \cdot \Delta pH - \Delta[lactate]$) of approximately zero. Thus, the observed acid–base imbalance can be explained solely by changes in plasma [lactic acid]. It is probable, however, that the characteristics of blood sampled within 1 min of the turtle reaching the surface may have underestimated the true acid–base imbalance, particularly the respiratory component of that imbalance. Visual observation indicated the average breathing frequency of turtles increased from approximately 1–2 breaths/min pre-trawl to 11 breaths/min post-trawl, representing a 9- to 10-fold increase in breathing frequency. It is possible, therefore, that some of the CO_2 retained during submergence or resulting from HCO_3^- buffering of metabolic protons, was released to the atmosphere once the turtle reached the surface. This would have reduced blood pCO_2, increased the $[HCO_3^-]/pCO_2$ ratio, and elevated blood pH. Given the magnitude of the observed imbalance, complete recovery of acid–base homeostasis in Kemp's ridley turtles may have required 7–9 hr (Lutz and Dunbar-Cooper, 1987).

The pre-trawl blood characteristics of Kemp's ridley turtles are comparable to data reported for other sea turtle species (Butler *et al.*, 1984; Wood *et al.*, 1984; Lutz and Bentley, 1985; Lutz and Dunbar-Cooper, 1987; Lutz *et al.*, 1989), with a caveat regarding the precision of our temperature-corrected blood gas/pH data. Animal transport to the trawl test site may have been a source of stress to the turtles, in as much as the observed decrease in plasma $[Cl^-]$ post-transport is consistent with renal compensation of extracellular acidosis. During the trawl test itself, renal mechanisms for regulating acid–base balance would not be expected to be significant because of the short test duration (i.e., minutes). A generalized cellular response to the trawl-induced acid–base imbalance is suggested, however, by the increase in plasma $[K^+]$ post-trawl.

The lactic acidosis observed in Kemp's ridley turtles under the present conditions had a rapid onset (after $\leqslant 7.3$ min of submergence), when compared to data on forced submergence of restrained/confined sea turtles. Berkson (1966), for example, found no significant change in blood [lactate] during the first 30 min of forced submergence of restrained green turtles. Lutz and Bentley (1985) reported a blood [lactate] comparable to the present post-trawl value (~10 mM) after 30 min of forced submergence of confined loggerhead turtles. Hochachka *et al.* (1975) reported a [lactate] of >35 mM after 2 hr of forced submergence of confined green turtles. The present data for Kemp's ridley turtles are in good agreement, however, with data measured in a freely-diving green turtle by Wood *et al.* (1984). Those authors obtained blood samples via an indwelling catheter from a tethered animal during a voluntary 15-min dive in open water. During the dive, blood pH declined from 7.66 to 7.21 and blood [lactate] increased from 0.6 to 10.4 mM. Wood *et al.* (1984) noted the turtle swam vigorously during most of the dive. Videotape analyses revealed the Kemp's ridley turtles used in our tests exhibited vigorous swimming motions upon release into the mouth of the trawl, presumably to prevent capture in the trawl. Trawling speed during the

Table 3. Effects of handling on the respiratory and acid–base status (mean ± SE, $N = 3$) of Kemp's ridley sea turtles at 30°C

Characteristic	Pre-handling	Post-handling	Difference (Post-Pre)
Blood pH	7.47 ± 0.05	7.41 ± 0.05	−0.07 ± 0.04*
Blood pCO_2, torr	38.4 ± 1.3	43.5 ± 1.5	5.1 ± 2.4*
Blood $[HCO_3^-]$, mM	31.1 ± 4.9	29.4 ± 5.2	−1.7 ± 1.1*

*Not significantly different from zero (paired t-test, $P > 0.05$).

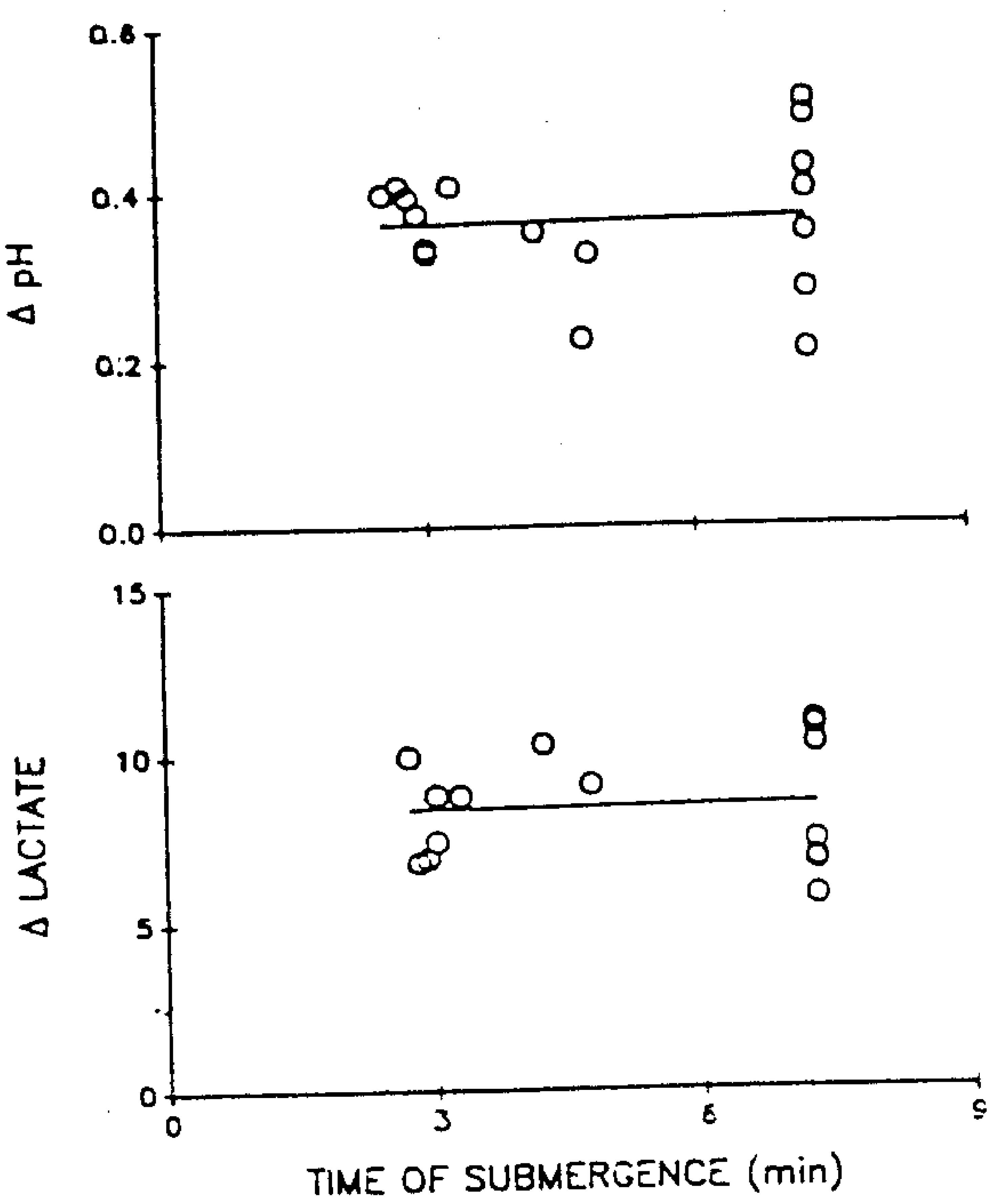

Fig. 1. Changes in blood pH and plasma [lactate] versus submergence time of Kemp's ridley sea turtles at 27°C.

present study was 128 cm/sec, which is considerably faster than the maximum swimming speed reported for Kemp's ridley turtles of the size of our test animals (Stabenau, 1988). Thus, differences in the dynamic responses of blood pH and lactate between studies conducted using restrained/confined sea turtles versus unrestrained animals in open water probably are attributable to differences in the level of apneic activity while submerged.

Many reptiles rely primarily on anaerobic metabolism during intensive activity bursts (Glass and Wood, 1983). This may hold true for sea turtles as well. Green turtles exercising on land, for example, exhibit a regular pattern of apneic activity and then quiescent recovery (Jackson and Prange, 1979). Jackson and Prange (1979) found evidence of lactic acidosis in green turtles after 20 min of this behavior, consistent with utilization of anaerobic metabolism during the activity bursts. Thus, our trawl tests probably incorporated two interdependent and confounding stressors which acted in concert to induce the observed lactic acidosis: a brief bout of involuntary submergence and a burst of vigorous apneic activity. Incidental capture of wild sea turtles in commercial trawls would be expected to involve similar stresses. We will not attempt to extrapolate our results to the changes in blood pH and lactate that may occur in captured sea turtles during the typical 1- to 6-hr shrimp trawl tows in the Gulf of Mexico/Atlantic Ocean (Henwood and Stuntz, 1987).

Sea turtles must be able to tolerate periods of apnea while submerged and still meet the metabolic demands of physical activity during such periods. These metabolic demands may be considerable in some instances, especially when underwater flight-to-escape strategies are evoked to avoid capture by predators (or trawls). The ability of sea turtles to tolerate tissue acidosis and anoxia (Berkson, 1966; Lutz and Bentley, 1985) would represent major advantages in this regard. The present data demonstrate that short-term forced diving coupled with vigorous apneic swimming is beyond the aerobic capacity of Kemp's ridley turtles. Whether the metabolic demands associated with voluntary diving routinely exceed the aerobic capacity of sea turtles is unclear from the existing literature and deserves further research.

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